Federal Democratic Republic of Ethiopia

EDICT OF GOVERNMENT

In order to promote public education and public safety, equal justice for all, a better informed citizenry, the rule of law, world trade and world peace, this legal document is hereby made available on a noncommercial basis, as it is the right of all humans to know and speak the laws that govern them.

ET ISO 7366 (1987) (English): Animal and vegetable fats and oils -- Determination of 1-monoglycerides and free glycerol contents

ISO INSIDE

BLANK PAGE

BRUTUM FULMEN

IGNORANTIA JURIS NON EXCUSAT

PROTECTED BY COPYRIGHT

ETHIOPIAN
STANDARD

ES ISO 7366:2012

First edition

Animal and vegetable fats and oils - Determination of 1-monoglycerides and free glycerol contents

(Identical with ISO 7366:1987)

ICS: 67.200.10

Published by Ethiopian Standards Agency
©ESA

Foreword

This Ethiopian Standard has been prepared under the direction of the Technical Committee for Animal and vegetables fats and oils (TC 23) and published by the Ethiopian Standards Agency (ESA).

The standard is identical with ISO :198 published by International Organization for Standardization (ISO).

For the purpose of this Ethiopian Standard, the adopted text shall be modified as follows.

- The phrase “International Standard” shall be read as “Ethiopian Standard”; and
- A full stop (.) shall substitute comma (,) as decimal marker.

Animal and vegetable fats and oils — Determination of 1-monoglycerides and free glycerol contents

1 Scope

This International Standard specifies a method for the determination of 1-monoglycerides content and of free glycerol content consecutively on the same test portion.

2 Field of application

It is applicable to animal and vegetable fats and oils and to interesterified products of oils, fats and fatty acids with glycerol.

It is not applicable when the sample contains

a) in addition to 1-monoglycerides : chloroform-soluble polyhydric substances with two or more adjacent hydroxyl groups;

b) in addition to free glycerol : water-soluble polyhydric substances with two or more adjacent hydroxyl groups.

3 Reference

ISO 5555, *Animal and vegetables fats and oils — Sampling.*

4 Principle

Dissolution of a test portion in chloroform. Extraction of free glycerol from this solution with acetic acid solution. Oxidation of 1-monoglycerides in the chloroform solution by an excess of periodic acid solution. Addition of potassium iodide and titration of the liberated iodine with a sodium thiosulfate standard volumetric solution.

Oxidation of free glycerol in the aqueous solution by an excess of periodic acid solution. Addition of potassium iodide and titration of the liberated iodine with a sodium thiosulfate standard volumetric solution.

5 Reagents

All reagents shall be of recognized analytical grade. The water used shall be distilled water or water of at least equivalent purity.

5.1 Chloroform.

5.2 Acetic acid, 5 % (V/V) solution.

5.3 Periodic acid, 2,7 g/l solution.

Weigh 2,7 g of periodic acid (H_5IO_6) in a 1 litre volumetric flask and dissolve in 50 ml of water. Make up to the mark with glacial acetic acid and mix thoroughly. Store in the dark.

5.4 Potassium iodide, 150 g/l solution, not containing free iodine or iodates.

5.5 Sodium thiosulfate, standard volumetric solution, $c(Na_2S_2O_3) = 0{,}1$ mol/l.

5.6 Starch, indicator solution, 10 g/l.

Dissolve 1 g of soluble starch in 100 ml of water by stirring and heating. Add 0,1 g of salicylic acid to preserve the indicator solution and boil for 3 min. Cool to room temperature.

6 Apparatus

Usual laboratory equipment and in particular

6.1 Conical flasks, of capacity 500 ml, with ground-glass stoppers.

6.2 Magnetic stirrer.

7 Sampling

See ISO 5555.

8 Procedure

8.1 Preparation of the test sample

8.1.1 Solid samples in flake or in powder form

Mix the sample thoroughly without melting.

©ESA

8.1.2 Other solid or semi-solid samples

Melt the sample at not more than 10 °C above its melting point, mix thoroughly.

CAUTION — The sample shall not be subjected to excessive temperatures. At temperatures above 80 °C the 1-monoglycerides content may decrease as a result of interesterification and/or intraesterification.

8.1.3 Liquid samples

Mix the sample thoroughly.

8.2 Test portion

Weigh accurately into a 50 ml beaker the appropriate mass of the test portion as indicated in the table.

Table

Expected content of 1-monoglycerides or of glycerol	Approximate mass of the test portion	Weighing accuracy
% (*m*/*m*)	g	g
100	0,3	0,000 1
75	0,4	0,000 1
50	0,6	0,000 1
40	0,7	0,000 1
30	1,0	0,001
20	1,5	0,001
15	2,5	0,001
10	3,0	0,001
5	6,0	0,001
3 or less	10,0	0,001

NOTE — If the contents of 1-monoglycerides and glycerol are very different and therefore an intermediate test portion mass is not possible, two test portions are necessary, one for the 1-monoglycerides and the other for the glycerol.

8.3 Extraction

8.3.1 Take three separating funnels of capacity 250 ml and label them 1, 2 and 3. Use separating funnel 1 to collect aqueous solutions and separating funnels 2 and 3 for washing the solutions. When shaking is required, this is performed vigorously for about 1 min, releasing pressure from time to time through the tap and avoiding the formation of emulsions.

Take two volumetric flasks of capacity 100 ml and label them A and B. Use flask A for the chloroform solution of 1-monoglycerides and flask B for the aqueous solution of glycerol.

8.3.2 Dissolve the test portion (8.2) in chloroform (5.1), dispersing any free glycerol. Pour quantitatively into separating funnel 1, using successive small quantities of chloroform to aid the transfer until the volume reaches 45 to 50 ml.

8.3.3 Rinse the used 50 ml beaker with about 25 ml of acetic acid solution (5.2) to dissolve any remaining glycerol. Transfer the rinsings to separating funnel 1. Stopper, shake and allow to separate. Run the chloroform (lower) layer into separating funnel 2.

Wash the 50 ml beaker with 25 ml of acetic acid solution and pour into separating funnel 2. Stopper, shake and allow to separate. Run the chloroform layer into separating funnel 3 and run the aqueous solution into separating funnel 1.

8.3.4 Wash the beaker and separating funnel 2 with a further 25 ml of acetic acid solution and run this into separating funnel 3. Stopper, shake and allow to separate. Run the chloroform layer into the 100 ml volumetric flask A. Run the aqueous layer into separating funnel 1.

Rinse separating funnels 2 and 3 successively with two 20 ml volumes of chloroform and run the chloroform into separating funnel 1. Stopper, shake and allow to separate.

8.3.5 Run the chloroform layer into the 100 ml volumetric flask A and make up to volume with chloroform. Stopper and mix.

8.3.6 Run the aqueous layer into the 100 ml volumetric flask B. Rinse the separating funnel 1 with acetic acid solution, add the rinsings to flask B and make up to volume with acetic acid solution. Stopper and mix.

8.4 Determination of 1-monoglycerides

Using a pipette, transfer 50 ml of the chloroform solution from flask A (8.3.5) into the 500 ml conical flask (6.1), add 50 ml of periodic acid solution (5.3) with a pipette, mix and stopper.

Allow to stand for 30 min in the dark.

Carry out a blank test under the same conditions, using 50 ml of chloroform (5.1) and 50 ml of periodic acid solution (5.3).

After the 30 min period add 20 ml of potassium iodide solution (5.4) both to the test solution and to the blank. Stopper the flasks, mix and allow to stand for 1 min longer.

Add 100 ml of water to each.

Titrate with sodium thiosulfate standard volumetric solution (5.5) whilst stirring continuously using the magnetic stirrer (6.2) in order to ensure thorough mixing. Towards the end of the titration, when the brown iodine colour has almost disappeared from the aqueous layer, add about 2 ml of starch solution (5.6) as indicator. Continue the titration dropwise until the end-point is reached (disappearance of the blue iodo-starch colour from the aqueous layer). Vigorous agitation is essential for complete removal of iodine from the chloroform layer.

If the volume used to titrate the test solution is not more than 80 % of the volume used to titrate the blank, repeat the determination using a smaller test portion or taking a smaller aliquot of the chloroform solution (to ensure an adequate excess of periodic acid).

8.5 Determination of free glycerol

Filter the aqueous solution from flask B (8.3.6).

Using a pipette, transfer 50 ml of the filtrate into a 500 ml conical flask (6.1), add 50 ml of periodic acid solution (5.3) with a pipette, mix and stopper.

Allow to stand for 30 min in the dark.

Carry out a blank test under the same conditions, using 50 ml of acetic acid solution (5.2) and 50 ml of periodic acid solution (5.3).

After the 30 min period add 20 ml of potassium iodide solution (5.4) both to the test solution and to the blank. Stopper the flasks, mix and allow to stand for 1 min longer.

Add 100 ml of water to each.

Titrate with sodium thiosulfate standard volumetric solution (5.5) adding 2 ml of starch solution (5.6) when the brown colour of the iodine has almost disappeared.

If the volume used to titrate the test solution is not more than 80 % of the volume used to titrate the blank, repeat the determination using a smaller test portion or taking a smaller aliquot of the aqueous solution (to ensure an adequate excess of periodic acid).

8.6 Number of determinations

Carry out two determinations on the same test sample.

9 Expression of results

9.1 Method of calculation and formulae

9.1.1 Calculation of 1-monoglycerides content

The 1-monoglycerides content, expressed as a percentage by mass, is equal to

$$\frac{(V_0 - V_1)\, crM_r}{20\, m}$$

where

V_0 is the volume, in millilitres, of the sodium thiosulfate standard volumetric solution required for the titration of the blank;

V_1 is the volume, in millilitres, of the sodium thiosulfate standard volumetric solution required for the titration of the test solution;

c is the concentration, expressed in moles per litre, of the sodium thiosulfate standard volumetric solution;

M_r is the relative molecular mass of a particular 1-monoglyceride, the choice depending on the composition of the fatty acids (when the results are expressed as glyceryl monostearate, $M_r = 358$);

m is the mass, in grams, of the test portion;

r is the ratio of the volume (100 ml) of the chloroform phase to the volume (50 ml or less) of this phase taken for the determination.

Express the result to one decimal place.

9.1.2 Calculation of free glycerol content

The free glycerol content, expressed as a percentage by mass, is equal to

$$\frac{(V_2 - V_3)\, crM_r}{40\, m}$$

where

V_2 is the volume, in millilitres, of the sodium thiosulfate standard volumetric solution required for the titration of the blank;

V_3 is the volume, in millilitres, of the sodium thiosulfate standard volumetric solution required for the titration of the test solution;

c is the concentration, expressed in moles per litre, of the sodium thiosulfate standard volumetric solution;

M_r is the relative molecular mass of glycerol ($M_r = 92{,}1$);

m is the mass, in grams, of the test portion;

r is the ratio of the volume (100 ml) of the aqueous phase to the volume of this phase (50 ml or less) taken for the determination.

Express the result to one decimal place.

9.2 Repeatability

The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst under the same conditions on the same test sample shall not exceed the values given in 9.2.1 and 9.2.2.

9.2.1 For 1-monoglycerides

0,5 % (*m*/*m*) at contents from 15 to 25 % (*m*/*m*)

2 % (relative) of the average value at contents from 25 to 50 % (*m*/*m*)

1 % (*m*/*m*) at contents greater than 50 % (*m*/*m*)

9.2.2 For free glycerol

0,1 % (*m*/*m*)

10 Test report

The test report shall show the method used and the results obtained. It shall also mention any operating details not specified in this International Standard, or regarded as optional, together with details of any incidents likely to have influenced the results.

The test report shall include all the information necessary for the complete identification of the sample.

የኢትዮጵያ የደረጃዎች ኤጀንሲ
Ethiopian Standards Agency

Organization and Objectives

The Ethiopian Standards Agency (ESA) is the national standards body of Ethiopia established in 2010 based on regulation No. 193/2010.ESA is established due to the restructuring of Quality and Standards Authority of Ethiopia (QSAE) which was established in 1970.

ESA's objectives are:-

- Develop Ethiopian standards and establish a system that enable to check weather goods and services are in compliance with the required standards,
- Facilitate the country's technology transfer through the use of standards,
- Develop national standards for local products and services so as to make them competitive in the international market.

Ethiopian Standards

The Ethiopian Standards are developed by national technical committees which are composed of different stakeholders consisting of educational Institutions, research institutes, government organizations, certification, inspection, and testing organizations, regulatory bodies, consumer association etc. The requirements and/ or recommendations contained in Ethiopian Standards are consensus based that reflects the interest of the TC representatives and also of comments received from the public and other sources. Ethiopian Standards are approved by the National Standardization Council and are kept under continuous review after publication and updated regularly to take account of latest scientific and technological changes. Orders for all Ethiopian Standards, International Standard and ASTM standards, including electronic versions, should be addressed to the Documentation and Publication Team at the Head office and Branch (Liaisons) offices. A catalogue of Ethiopian Standards is also available freely and can be accessed in from our website.

ESA has the copyright of all its publications. No part of these publications may be reproduced in any form without the prior permission in writing of ESA.

International Involvement

ESA, representing Ethiopia, is a member of the International Organization for Standardization (ISO), and Codex Alimentarius Commission (CODEX). It also maintains close working relations with the international Electro-technical Commission (IEC) and American Society for Testing and Materials (ASTM).It is a founding member of the African Regional Organization for standardization (ARSO).

More Information?
Contact us at the following address.
The Head Office of ESA is at Addis Ababa.

☎ 011- 646 06 85, 011- 646 05 65
📠 011-646 08 80
✉ 2310 Addis Ababa, Ethiopia
E-mail: info@ethiostandards.org,
Website: www.ethiostandards.org

Standard Mark

DRAFT